

ORIGINAL ARTICLE

Infection of infants with human herpesvirus type 6 may be associated with reduced allergic sensitization and T-helper type 2 development

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Summary

Background Epidemiological studies point to an inverse relationship between microbial exposure and the prevalence of allergic diseases. The underlying mechanism for this observation remains largely unknown, as well as the nature of the microbes involved.

Objective To investigate the effects of early infection with human herpesviruses (HHVs) on IgE formation and T-helper type 2 (Th2) development in infants.

Methods Serum was collected from children aged 18 months and assessed for IgE to common allergens and IgG to five common herpesviruses. Cord blood plasmacytoid dendritic cells (pDC) were exposed to HHV type 6 *in vitro* and mixed with allogeneic cord blood CD4⁺ T cells. Cytokine levels were determined by ELISA and by flow cytometry.

Results We found that children seropositive at 18 months of age to HHV type 6 were significantly less often IgE sensitized than seronegative children [odds ratio (OR): 0.08, 95% confidence interval (CI): 0.009–0.68]. HHV type 6 also decreased the production of the Th2-associated cytokines IL-5 and IL-13 by CD4⁺ T cells when co-cultured with allogeneic cord blood pDC. This was associated with an increased production of IFN- α by pDC exposed to HHV type 6.

Conclusion These data indicate that an early childhood infection with HHV type 6 could down-regulate Th2 responses and reduce IgE formation to common allergens in a young child.

Keywords allergy, human herpesvirus type 6, IgE, infants, interferon, Th2

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Introduction

The prevalence of allergic diseases has increased dramatically in the developed world during the last decades. One theory, the hygiene hypothesis, postulates that a reduced incidence of childhood infections might be one important contributing factor. The hygiene hypothesis was initially based on the observation that poverty as well as large numbers of elder siblings protected infants from allergic development [1]. Certain viral infections have been associated with a reduced incidence of IgE sensitization and/or allergy. Epidemiological studies show that a previous hepatitis A virus infection is inversely associated with atopy [2, 3]. Childhood infection with certain viruses of the herpes virus family, i.e. Epstein-Barr virus (EBV), also correlates with a reduced incidence of allergic sensitization [4, 5].

Herpesviruses are among the most common human viral pathogens. Human herpesvirus type 6 (HHV-6) is often the first herpesvirus encountered by the young

child, and infection generally occurs during the first year of life [6, 7]. HHV-6 causes *exanthema subitum*, a benign disease in young children characterized by high fever for 3–5 days. In Sweden, 60% of children become infected by HHV-6 before 1 year of age and more than 90% of adults have anti-HHV-6 antibodies [8]. HHV-6 replicates most efficiently in activated T cells but can infect all myeloid and lymphoid cells. Once an individual has been infected, the virus is continuously shed in the saliva, which is believed to serve as the main route of transmission.

Plasmacytoid dendritic cells (pDC) are instrumental in the anti-viral defence. These cells are the main IFN- α -producing cells in the body [9] and are rapidly activated by many HHVs [10–12]. Intriguingly, pDC appear to protect against allergic sensitization and IgE-mediated inflammation in a mouse model [13]. Human pDC might, through their secretion of IFN- α , deviate a Th2-dominated response into a more Th1-dominated one *in vitro* [14].

In this study, we examined the association between seropositivity for five different herpesviruses, EBV, varicella zoster virus (VZV), cytomegalovirus (CMV), herpes simplex virus (HSV) and HHV-6 to the incidence of allergic sensitization and allergic manifestations in 18-month-old children. We also evaluated the functional effects of two selected herpesviruses, HHV-6 and CMV, on cord blood leucocytes. Our data indicate that HHV-6 exposure could be associated with a reduced incidence of Th2 development and allergic sensitization in infants.

Methods

Subjects

Sixty-four healthy Swedish infants born in 2001–2003 at the Sahlgrenska University Hospital (Göteborg, Sweden) were recruited. These children formed the prospective Immunoflora-birth cohort study, investigating the relation between gut bacterial colonization, immune system development and allergy. The majority (>80%) of children were selected for a positive parental history of allergy in order to obtain sufficient numbers of children developing allergic sensitization and allergic disease. Demographic characteristics of this cohort have been reported previously [15]. Informed consent was obtained from the parents. The study was conducted according to the declaration of Helsinki principles and approved by the Human Research Ethics Committee of the Medical Faculty, University of Gothenburg, Sweden. All children completed the study and were followed up to 18 months of age.

Cord blood used in the *in vitro* studies was obtained from unselected healthy infants.

Assessment of herpesvirus infection

Plasma was prepared from heparinized blood samples collected from 18-month-old children. We obtained samples from 59 of the 64 children. The plasma samples were diluted 1/100 and analysed for the presence of virus-specific IgG to EBV, HSV, VZV, CMV and HHV-6. EBV antibodies were detected using the Anti-EBV IgG ELISA (Biotest, Dreieich, Germany). HHV-6 antibodies were detected using the HHV-6 IgG EIA (Biotrin International Ltd, Dublin, Ireland) and by immunofluorescence staining of HHV-6-infected cells as described [8]. The latter analysis was performed by the routine Clinical Virology laboratory at Sahlgrenska University hospital, Gothenburg, Sweden. Negative and positive control sera verified by the external quality assurance in laboratory medicine in Sweden (Equalis AB, Uppsala, Sweden) were included in each assay.

IgG to VZV, CMV and HSV were analysed by in-house ELISA. The complete membrane protein fractions from each of these viruses were prepared from infected cell cultures, as described in [16–18], and used as coating

antigens. Plasma samples in duplicates were added to the antigen-coated wells, followed by horseradish peroxidase-conjugated affinity-purified goat anti-human IgG (Southern Biotechnology Ass., Birmingham, AL, USA) and 1 mg/mL *O*-phenylenediamine dihydrochloride (Sigma, St Louis, MO, USA) in 0.1 M citrate buffer pH 4.5 containing 0.025% H₂O₂. Development was stopped with 2 M H₂SO₄ and the absorbance measured at 490 nm. Negative and positive control sera verified by external quality assurance in laboratory medicine in Sweden (Equalis AB) were included in each assay.

Clinical evaluation

Structured telephone interviews with the parents were performed when the children were 6- and 12-month-old probing feeding practice, family and living conditions, infections and other type of diseases. In addition, the parents were instructed to contact the study nurse upon the appearance of any symptoms that could indicate allergy in the child. In such cases, an appointment was arranged with one of the paediatric allergist and/or the specialist in paediatric gastroenterology. At 18 months, all children were examined by a paediatric allergist. A clinical examination and a structured interview were performed. A capillary blood sample was obtained from 62 of the 64 children and analysed for specific IgE to common allergens (ImmunoCap Phadiatop[®] and ImmunoCap Fx5; Phadia, Uppsala, Sweden). If any of the screening tests were positive, allergen-specific IgE tests were performed to the specific allergens (ImmunoCap to birch, timothy, mugwort, dog, cat, horse, house dust mite, cladosporium, cow's milk, hen's egg, fish, wheat, peanut and soy; Phadia). An allergen-specific IgE test ≥ 0.35 kU/L was considered positive. Skin prick test (SPT; ALK-Abello AS, Hørsholm, Denmark) was used when investigating suspected food allergies, and included the suspected food, positive control (histamine 10 mg/mL) and negative control (allergen diluent). A standard extract (Soluprick[®] SQ, ALK-Abello AS) was used in accordance with European guidelines [19] and a weal diameter ≥ 3 mm was considered a positive SPT response. A diagnosis of food allergy, eczema, asthma and allergic rhinoconjunctivitis (ARC) was based on medical records, the structured interview and on clinical signs of allergic manifestations *Food allergy*: An immediate- (≤ 2 h) or late-onset (> 2 h) reaction after ingestion of the specific food, followed by a clear and prompt clinical improvement when eliminating the suspected food allergen. The diagnosis was, when possible, supported by at least one provocation test, *Eczema*: William's criteria were used [20], *Asthma*: At least three episodes of viral wheeze together with wheeze/symptoms in between infections and/or other manifestations of allergy, or chronic/persistent wheeze with heavy breathing or cough for at least 1 month and *ARC*: Symptoms from the eyes

and/or nose on exposure to pollen or animals together with a positive allergen-specific IgE test to this specific allergen.

Virus

HHV-6 strain Z 29 (variant B) was kindly donated by Dr Helena Dahl, SMI, Stockholm. The virus was grown in MOLT-3 cells in Iscoves complete medium [Iscoves medium with 10% foetal bovine serum (Biological Industries, Kibbutz Beit Haemek, Israel), 3 µg/mL L-glutamine (Gibco, Invitrogen, Stockholm, Sweden), 0.1 mg/mL gentamycin sulphate (Essex Läkemedel AB, Stockholm, Sweden)] containing 5 µg/mL cortisone (Solucortef, UpJohn, Pfizer-AB, Sollentona, Sweden). After 2–3 weeks, the suspensions were centrifuged at 478 g. The virus-containing supernatants were then centrifuged for 1 h at 100 000 g. The viruses were resuspended in Iscoves complete medium. Viral content was determined by quantitative PCR. When indicated, the virus was inactivated at 2500 rad.

CMV was a kind gift from Prof Tomas Bergström, University of Gothenburg. Also, this virus was inactivated at 2500 rad.

Activation of plasmacytoid dendritic cells

Fresh cord blood pDC were isolated from cord blood mononuclear cells (CBMC) using the pDC isolation kit from Miltenyi Biotec (Auburn, CA, USA). The purity of the pDC obtained from CBMC was lower than when using adult blood, and varied from 60% to 90%. pDC were dispersed in 24-well plates (10^6 cells/well) in the presence or absence of HHV-6 or CMV (both used at 70 genome copies/pDC) in 1 mL Iscoves complete medium. Supernatants were collected after 24 h and frozen at -70°C until assayed for IFN- α content using an ELISA kit from PBL Biomedical Laboratories (Piscataway, NJ, USA). The IFN- α results were adjusted to the purity of the pDC preparations and corresponded to the secretion from 10^6 pDC.

Mixed lymphocyte reaction

CD4⁺ T cells were isolated from CBMC using the Dynal CD4⁺ Isolation Kit according to the manufacturer's instructions (Invitrogen AB, Stockholm, Sweden). CD4⁺ T cells (10^5) were cultured with allogeneic pDC (2×10^4) in 96-well flat-bottom Nunc tissue culture plates (Fisher Scientific AB, Västra Frölunda, Sweden) in Iscoves complete medium together with irradiated HHV-6 or CMV (70 genome copies/pDC). Supernatants were collected after 48 h and analysed for cytokine content. IL-13 levels were determined using an IL-13 DuoSet ELISA (R&D, Minneapolis, MN, USA). IFN- γ , TNF- α , IL-2, IL-4, IL-5 and IL-10 content were determined using the Human Th1/Th2 Cytokine Cytometric Bead Array Kit (BD Biosciences Pharmingen, San Diego, CA, USA). Cytokine-bound cytometric beads were analysed on a FACSanto2 flow cytometer and the data were analysed using the FACS array Software (BD Biosciences Pharmingen).

Statistical evaluations

Student's *t*-test, ANOVA with Bonferroni's multiple comparison test, Mann-Whitney test, Fisher's exact test and calculations of odds ratios (ORs) and 95% confidence intervals (CIs) were performed using GraphPad Prism version 5 for Window (GraphPad Software, San Diego, CA, USA).

Results

Assessment of the study cohort in relation to allergic diseases

All 64 recruited children completed the follow-up and underwent a clinical evaluation regarding the presence of allergic manifestations at the age of 18 months. This examination included a structured interview, clinical examination and evaluation of total and allergen-specific IgE. Forty-nine children (77%) were healthy. Fifteen children (23%) had clinical manifestations of allergy, out of which 12 had eczema, six had food allergy, four had asthma and two had ARC. Part of the demographic data of the cohort has been reported previously [15] and is summarized in Table 1. There were no significant differences between healthy and allergic children regarding gestation age, atopic heredity, breast feeding, older siblings, parental education or exposure to smoke or furry pets. However, there were significantly fewer girls among

Table 1. Demographic data of children in the cohort

	All (<i>n</i> = 64)	Healthy (<i>n</i> = 49)	Allergic disease [‡] (<i>n</i> = 15)	<i>P</i> -value [§]
Female, <i>n</i> (%)	34 (53)	30 (61)	4 (27)	0.04
Parental allergy*				
None, <i>n</i> (%)	12 (19)	11 (22)	1 (6.7)	NS
One parent, <i>n</i> (%)	39 (61)	27 (55)	12 (80)	NS
Two parents, <i>n</i> (%)	12 (19)	11 (22)	1 (6.7)	NS
Unknown, <i>n</i> (%)	1 (1.6)	0	1 (6.7)	NS
Breastfeeding at 6 months, <i>n</i> (%)	49 (77)	38 (78)	11 (73)	NS
Parent(s) smoking, <i>n</i> (%)	4 (6.2)	4 (8)	0	NS
Furred pets at home, <i>n</i> (%)	6 (9.3)	5 (10)	1 (6.7)	NS
Older siblings, <i>n</i> (%)	35 (55)	28 (57)	7 (47)	NS
Parental education				
High [†] , <i>n</i> (%)	55 (86)	42 (86)	13 (87)	NS
Gestation age (weeks)				
38–42	61 (95)	46 (94)	15 (100)	NS
<38	3 (4.7)	3 (6.1)	0	NS

*Parental history of asthma, ARC or eczema.

[†]High education depicts families where at least one parent has a university education.

[‡]Eczema, food allergy, asthma or ARC.

[§]Statistical difference between healthy children and children with allergic disease (Fisher's exact test).

NS, not significant; ARC, allergic rhinoconjunctivitis.

Table 2. Allergic sensitization in the study cohort evaluated at 18 months of age

	All	Healthy	Allergic disease [‡]	P-value [§]
Total IgE				
µg/mL, median (range)	14 (2–1900)	11 (2–520)	14 (3–1900)	NS
Allergen-specific IgE				
Inhaled antigens*, ratio (%)	2/61 (3)	0/47 (0)	2/14 (14)	0.05
Food antigens [†] , ratio (%)	12/62 (19)	5/47 (11)	7/15 (47)	0.006

*Screening for allergen-specific IgE to birch, timothy, mugwort, dog, cat, horse, house dust mite and cladosporium. Data on allergen-specific IgE to inhaled antigens was available from 62 of the 64 children included in the study.

[†]Screening for allergen-specific IgE to cow's milk, hen's egg, fish, wheat, peanut and soy.

[‡]Eczema, food allergy, asthma or ARC.

[§]Statistical differences between healthy children and children with allergic disease using Mann–Whitney test for total IgE and Fisher's exact test for allergen-specific IgE.

NS, not significant.

the allergic children. There was no difference in total IgE levels between the groups, but a larger proportion of the children with allergic manifestations showed a positive allergen-specific IgE test to both inhaled antigens and food antigens than healthy children (Table 2).

Herpesvirus infection protects children from allergen sensitization

Plasma was obtained at 18 months of age from 59 of the 64 children included in the study and screened for IgG reactivity to HSV, CMV, EBV, VZV and HHV-6. Five percent of the children were seropositive for EBV, 8% for HSV, 17% for VZV, 37% for CMV and 45% for HHV-6. Twenty-four percent of the children were negative for all five viral agents, 51% were seropositive for one agent, 15% for two, 8% for three and 2% for four of these agents.

The occurrence of allergen-specific IgE was assessed in 56 of the children. Nineteen percent of these children were found to have developed allergen-specific IgE. The incidence of allergic sensitization was significantly lower in children infected with at least one herpes virus (Table 3), with an OR of 0.13. However, the incidence of allergic disease did not differ significantly between the herpes virus-infected children and those who had not been exposed to these viruses (Table 3).

Human herpesvirus-6 infection protects children from immunoglobulin E sensitization

To delineate if exposure to any particular herpesvirus was associated with a lower prevalence of IgE sensitization, we analysed the incidence of allergen-specific IgE in the study cohort in relation to the herpesvirus infection status. When we compared HHV-6 seropositive and HHV-6 seronegative children, we found that HHV-6 seropositivity was statistically associated with a lower incidence of IgE sensitization at an OR of 0.08 (Table 4). This association was even stronger if we compared the 26 HHV-6-

Table 3. Herpesvirus infection is associated with a lower incidence of IgE sensitization in 18-month-old children

	IgE sensitization* (n = 56) [‡]	Allergic disease [†] (n = 59)
Herpesvirus-infected children	5/44 (11%)	9/45 (20%)
Herpesvirus-negative children	6/12 (50%)	5/14 (36%)
P-value [§]	< 0.01	0.28
OR (95% CI)	0.13 (0.03–0.56)	0.45 (0.12–1.67)

*Presence of IgE antibodies to birch, timothy, mugwort, dog, cat, horse, house dust mite, cladosporium, cow's milk, hen's egg, fish, wheat, peanut or soy in serum samples collected at 18 months of age.

[†]Eczema, food allergy, asthma and/or ARC.

[‡]Data on IgE sensitization was available from 56 of the 59 children that were examined for herpesvirus infections.

[§]Statistical difference between herpesvirus-infected children and seronegative children (Fisher's exact test).

OR, odds ratio; CI, confidence interval; ARC, allergic rhinoconjunctivitis.

Table 4. HHV-6 infection is associated with a lower incidence of allergic sensitization in 18-month-old children

	IgE sensitization* (n = 56) [‡]	Allergic disease [†] (n = 59)
HHV-6-seropositive children	1/26 (4%)	4/27 (15%)
HHV-6-seronegative children	10/30 (33%)	10/32 (31%)
P-value [§]	< 0.01	0.22
OR (95% CI)	0.08 (0.009–0.68)	0.38 (0.10–1.40)

*Presence of IgE antibodies to birch, timothy, mugwort, dog, cat, horse, house dust mite, cladosporium, cow's milk, hen's egg, fish, wheat, peanut or soy in serum samples collected at 18 months of age.

[†]Eczema, food allergy, asthma and/or ARC.

[‡]Data on IgE sensitization was available from 56 of the 59 children that were examined for herpesvirus infections.

[§]Statistical difference between HHV-6-infected children and seronegative children.

OR, odds ratio; CI, confidence interval; HHV, human herpesvirus.

infected children with the 13 children who were not infected with any of the five herpesviruses ($P < 0.003$, $OR = 0.047$, 95% $CI = 0.005-0.46$). No such correlation was observed when we compared CMV seropositive and CMV seronegative children ($P = 0.51$, $OR = 0.56$, 95% $CI = 0.13-2.41$, data not shown) nor when we compared children who were HHV-6 negative but positive to at least one of the other herpesviruses with children who were not infected with any of the five herpesviruses (data not shown). The low incidences of seropositivity to EBV, HSV and VZV at 18 months of age in combination with the limited size of the study group precluded us from performing statistical analysis of possible relationships between these infections and IgE sensitization. The incidence of HHV-6 infection was similar in children born to allergic parents (39%) and in children with no family history of allergy (50%) ($P = 0.53$, Fisher's exact test). No significant reduction of allergic disease in the HHV-6-infected children was detected at 18 months of age.

Human herpesvirus-6 promotes strong interferon- α responses in cord blood plasmacytoid dendritic cells

pDC have been ascribed a central role in preventing allergic sensitization in a mouse asthma model [13]. The ability of pDC to prevent Th2 responses is linked to their secretion of the anti-viral cytokine IFN- α [14]. We have therefore investigated if HHV-6 could activate cord blood pDC to secrete IFN- α *in vitro*. HHV-6 induced a strong IFN- α response in pDC from newborn infants (Fig. 1). Further-

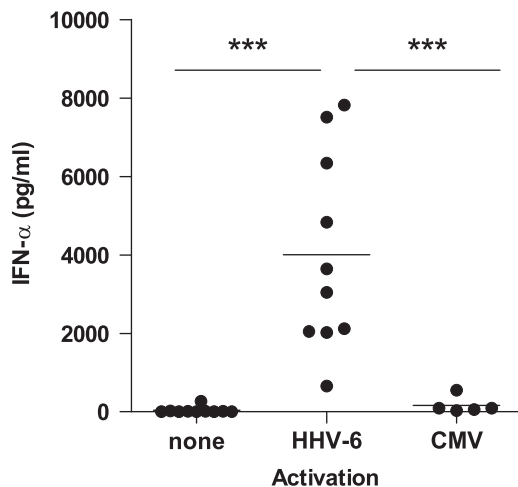


Fig. 1. Human herpesvirus type 6 (HHV-6) promotes IFN- α production by cord blood plasmacytoid dendritic cells (pDC). Cord blood pDC were incubated for 24 h in the presence or absence of HHV-6 or cytomegalovirus (CMV) and the IFN- α levels were measured in the supernatants by ELISA. Data are depicted as individual values and means with *** $P < 0.001$ using ANOVA with Bonferroni's multiple comparison test. $n = 10$ for HHV-6 and $n = 5$ for CMV.

more, HHV-6 exposure protected the pDC from apoptosis in culture. $40 \pm 7\%$ of HHV-6-exposed pDC were viable after 24 h in culture, as compared with $27 \pm 2\%$ of unexposed pDC (not shown).

Human herpesvirus-6 blocks T-helper type 2 responses in a mixed lymphocyte reaction using cord blood plasmacytoid dendritic cells and $CD4^+$ T cells

To investigate whether HHV-6 exposure of pDC could affect the subsequent T cell polarization, cord blood pDC were incubated with allogeneic cord blood $CD4^+$ T cells in the presence or absence of HHV-6. When culture supernatants were analysed for cytokine content, we found that T cells activated in the presence of HHV-6 produced significantly lower levels of the Th2 cytokines IL-5 (Fig. 2a) and IL-13 (Fig. 2b). At the same time, IL-10 production was significantly up-regulated (Fig. 2c) as well as the production of IFN- γ (Fig. 2d). No statistically significant changes in the levels of IL-2, or TNF- α were observed, and there were no measurable levels of IL-4 in any of the culture supernatants (not shown).

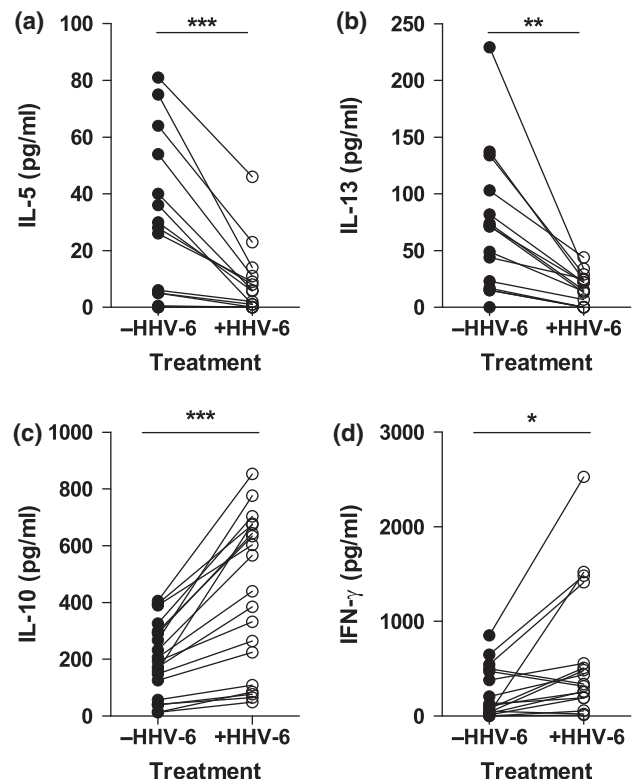


Fig. 2. Effect of human herpesvirus type 6 (HHV-6) on T cell cytokine responses. Cord blood plasmacytoid dendritic cells (pDC) were incubated with allogeneic cord blood $CD4^+$ T cells in the presence or absence of HHV-6. Supernatants were collected after 48 h and analysed for IL-5 (a), IL-13 (b), IL-10 (c) and IFN- γ (d) content. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ using Student's *t*-test. $n = 10-14$.

Effect of cytomegalovirus on cytokine responses by plasmacytoid dendritic cells and T cells

To assess whether the effects of CMV on pDC function and Th1/Th2 cytokine production were a universal effect of herpesvirus exposure, we performed a control experiment where pDC were incubated together with inactivated CMV. We found that CMV did not induce any enhanced IFN- α secretion by pDC (Fig. 1). However, inclusion of CMV in cultures of cord blood pDC and allogeneic CD4⁺ T cells significantly reduced the levels of secreted IL-5 and IL-13 (Figs 3a and b) in a fashion similar to that observed with HHV-6 (see Fig. 2). In contrast, CMV did not concomitantly enhance the levels of IL-10 and IFN- γ in culture supernatants (Figs 3c and d). Instead, there was a trend that CMV reduced the levels of IFN- γ (Fig. 3d).

Discussion

In this study, we show that HHV-6 infection is associated with a significantly reduced incidence of allergic sensitization in 18-month-old children. *In vitro*, we show that HHV-6-activated cord blood pDC produce IFN- α . In a mixed lymphocyte reaction, HHV-6-exposed pDC down-regulated

the production of the Th2-associated cytokines IL-5 and IL-13, and instead promoted the production of IL-10 and IFN- γ . These data imply that HHV-6 infection could prevent the development of Th2-associated immune responses.

The incidence of HHV-6 infection in the 18-month-old children included in the study cohort was 45%. This is lower than that reported previously in Swedish infants, where 84% were seropositive at 2 years of age [4]. Both the small study cohort presented in our study, the difference in age of the children examined (18 months vs. 2 years) as well as methodological differences should be taken into account when comparing these studies. In our study, we used a titre of 100 to depict a positive serological response, whereas other studies have used titres of 10 and 20 as cut-offs [4, 8]. Recent studies do, however, show that IF titres below 32 are not true positives but require confirmation either by detection of viral DNA, or by a later rise in antibody titre [21]. Thus, by using a high titre as cut-off, we ensured that not only are we dealing with true positive results but also we probably excluded children with very recent primary infection. The latter is intentional, as we envision that it takes a few weeks for a viral infection to impact on pre-existing IgE responses.

Our data indicate that HHV-6 infection is negatively associated with allergic sensitization in 18-month-old children. Similar associations between herpesviruses infections and allergic sensitization in young children have been made previously for EBV [4, 5, 22]. HHV-6 and EBV share several characteristics. Both viruses infect lymphocytes (T cells and B cells, respectively) and might thereby have a direct effect on the functionality of the adaptive immune system. Given the migratory nature of these cells, the virus-infected cells will be dispersed throughout the body. Infections caused by these two viruses require an intact and life-long Th1-driven cell-mediated immune response for their containment [23, 24]. In addition, both EBV and HHV-6 induce type I IFN production by adult pDC [10, 12].

We were not able to show any statistical significance between HHV-6 infection and allergic disease. However, the incidence of allergic sensitization is higher among children with allergic disease than among non-allergics [25–28] and allergic sensitization is known to be a risk factor for the later development of allergic disease [29].

Only 8% of the children in our study were found to be EBV seropositive at 18 months of age. This was considerably lower compared with the 26% incidence found in 2-year-old Swedish infants [4]. Two factors probably account for this difference; (i) a considerable number of children might be infected between 18 and 24 months of age; (ii) different studies use different methods to detect EBV-specific IgG antibodies, and these assays might have different sensitivities. In our case, a commercial ELISA test was used, whereas Nilsson et al. [4] used IF.

Given the limited number of children in the study and the low incidence of EBV, HSV and VZV infections

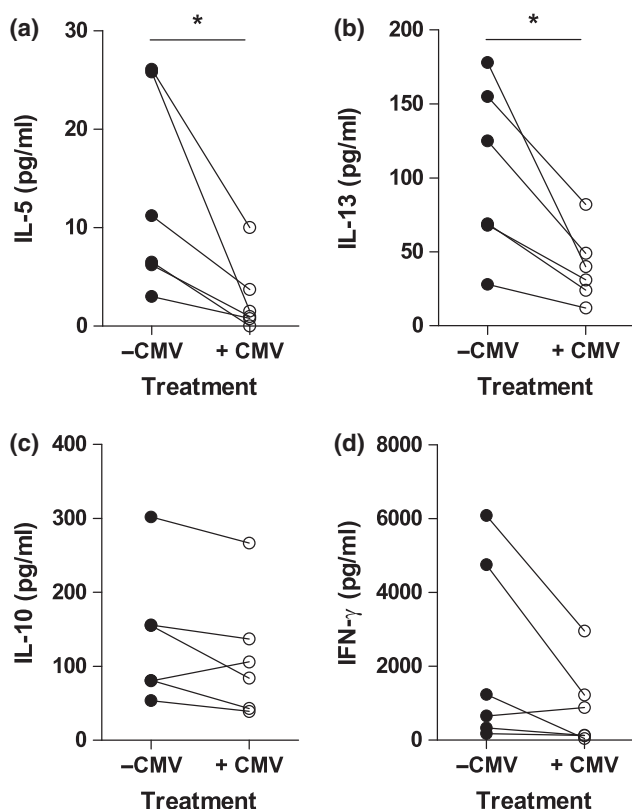


Fig. 3. Effect of cytomegalovirus (CMV) on T cell cytokine responses. Cord blood plasmacytoid dendritic cells (pDC) were incubated with allogeneic cord blood CD4⁺ T cells in the presence or absence of CMV. Supernatants were collected after 48 h and analysed for IL-5 (a), IL-13 (b), IL-10 (c) and IFN- γ (d) content ($n = 5$). * $P < 0.05$ using Student's t -test. $n = 5$.

unfortunately prevents us from predicting the role of these viruses in the development of atopy. To assess if these viruses have the same beneficial effect on the development allergic sensitization in 18-month-old children requires a significantly larger study group. The only two herpesviruses that were prevalent enough to allow any reliable statistical evaluations were HHV-6 and CMV. Of these, HHV-6 infection was clearly associated with a reduced incidence of allergic sensitization (both when compared with HHV-6 seronegatives and to those negative for all five herpesviruses examined) whereas CMV, as reported previously, was not [5, 30]. We thus propose that HHV-6 infection, by occurring so early in life (often before EBV infection), was able to modulate the developing immune system before 18 months of age.

The age of viral acquisition could be an important factor influencing the later development of allergy. Many epidemiological studies confirm the notion that events occurring early in childhood strongly influence the risk of allergy development later [31, 32]. This stresses the importance of examining the prevalence of allergic and infectious disorders in very young children. If the examination is performed in older children or in adults when a majority of individuals have already acquired most of these infections, the potentially beneficial role of an early herpesvirus exposure might no longer be apparent. For instance, no correlation between HHV-6 seropositivity and IgE sensitization is observed in 2-year-old Swedish children [4]. However, as mentioned before, these studies used a more generous detection limit for HHV-6 seropositivity.

A limitation to our study is the limited number of children that were included. However, given the fact that HHV-6 infection was associated with a significantly reduced incidence of allergic sensitization (including a convincing OR of 0.08) as well as reduced Th2 responses *in vitro*, it warrants our preliminary conclusion that HHV-6 infection could protect young children from developing allergic diseases. However, it is of course important to confirm our observation in large prospective studies where HHV-6 infection is evaluated at 18 months of age.

pDC are held to promote Th2 responses. However, following exposure to certain virus or TLR ligands *in vitro*, these cells produce high amounts of IFN- α that drives Th1 responses [14, 33–36]. This fits well with our results showing that HHV-6-exposed pDC, which secreted high levels of IFN- α , had a reduced ability to induce the production of Th2 cytokines such as IL-5 and IL-13 in responding T cells and instead induced elevated levels of IFN- γ . CMV, on the other hand, promoted neither IFN- α production by pDC nor subsequent IFN- γ responses by T cells, even though it did impact negatively on Th2 cytokine production. Given the lack of impact of CMV on allergic sensitization, we propose that IFN- α and IFN- γ production, rather than reduced Th2 responses alone, accounts for the potential protective capacity of HHV-6

against allergic sensitization. This is in line with a report showing that administration of IFN- α can block the induction of IgE and allergic symptoms in a murine model of allergic disease [37]. This relationship is also evident in humans as blood dendritic cell from allergic subjects have an impaired capacity to produce IFN- α [38]. In addition, the levels of allergen-specific IgE in cord blood are inversely correlated to the IFN- γ production by cord blood T cells [39].

Both IL-5 and IL-13 are associated with IgE production. IL-13, similar to IL-4 can, induce IgE switching in human B-cells *in vitro* [40, 41], and most likely also *in vivo*, given the correlation between polymorphisms in the IL-13 gene and serum levels of IgE [42]. IL-5 does not have direct effects on IgE switching but promotes eosinophil production in the bone marrow and is increased in clinical allergies [43]. Thus, our *in vitro* data showing that HHV-6-exposed human pDC had a decreased ability to induce IL-5 and IL-13 from CD4⁺ T cells may have an impact on both IgE production and symptoms.

In summary, we show that HHV-6 drives the production of IFN- α from pDC, which *in vitro* blocks Th2 T cell development. This is particularly interesting given the important role of pDC in preventing asthmatic reactions in rodents [13]. In young children, HHV-6 infection is associated with a reduced incidence of allergen-specific IgE in 18-month-old children compared with children not yet exposed to HHV-6. Collectively, our data suggest that HHV-6 might suppress Th2 immune responses, and thereby potentially protect against IgE sensitization.

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